Synchronization of the estrous during a short period, using a low dose of equine chorionic gonadotropin (eCG) in primiparous and multiparous ewes

Cordero-Mora, José L.[1]; Sánchez-Torres, Teresa[1]; Nieto-Aquino, Rafael[2*]; Salinas-Ríos, Teodulo[3]; Hernández-Bautista, Jorge[3]; Figueroa-Velasco, José L.[1]; Martínez-Aispuro, José A.[1]

[1] Colegio de Postgraduados, Recursos Genéticos y Productividad – Ganadería, C. P. 56264, Montecillo, Estado de México.
[2] Tecnológico Nacional de México, Instituto Tecnológico de Huejutla, Ingeniería en Agronomía con Especialidad en Zootecnia, C.P. 43000, Huejutla de Reyes, Hidalgo, México.
[3] Universidad Autónoma Benito Juarez de Oaxaca, Facultad de Medicina Veterinaría y Zootecnía, C. P. 68120, Oaxaca de Juárez, México.
* Correspondence: nietoaquinorafael@gmail.com

Citation: Cordero-Mora, J. L., Sánchez-Torres, T., Nieto-Aquino, R., Salinas-Ríos, T., Hernández-Bautista, J., Figueroa-Velasco, J. L., & Martínez-Aispuro, J. A. (2023). Synchronization of the estrous during a short period, using a low dose of equine chorionic gonadotropin (eCG) in primiparous and multiparous ewes. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i2.2263

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: April 30, 2022.
Accepted: January 12, 2023.
Published on-line: April 12, 2023.

Agro Productividad, 16(2). February. 2023. pp: 3-10.

ABSTRACT

Objective: To evaluate the reduction effect of the synchronized luteal phase and the eCG application in the reproductive variables and hormone profile response of primiparous and multiparous ewes.

Design/Methodology/Approach: The experimental design was completely random, with a 2×2×2 factorial arrangement. Based on their reproductive activity, the 81 specimens were divided into primiparous (n=38) and multiparous (n=43) ewes. The estrous and pregnancy stages were analyzed using the χ^2 test. An analysis of variance and the Tukey's mean comparison test were used to determine the start and the duration of the estrous. P_4 concentration was measured using the PROC MIXED which influenced the fixed effects of the treatment and the day, as well as their interaction.

Results: There was no difference between treatments, regarding the occurrence of the estrous; however, its start and duration were not impacted by the physiological state and the synchronized luteal phase. P_4 concentrations in plasm were higher in primiparous ewes than in multiparous ewes. The main effects did not impact the pregnancy and the prolificity rates.

Study Limitations/Implications: The variation in the start and the duration of the estrous was caused by the physiological reproductive state and the duration of the synchronized luteal phase (6 and 12 days). Therefore, these effects should be taken into account when the artificial insemination takes place at a fixed period.

Finding/Conclusions: The combination of the cronolone sponges with 100 UI of eCG during short periods (6 days) effectively synchronizes the estrous. P_4 concentrations in serum were higher in primiparous ewes, although these concentrations were not a determining factor in the increase of pregnancy and prolificity.

Keywords: Cronolone, estrous, sheep, progesterone, synchronization.

Image by Manfred Richter at Pixabay

INTRODUCTION

Several hormone methods have been developed to synchronize the estrous of ewes. They are mainly based on natural and synthetic progesterone, gonadotropins, prostaglandin $F_{2\alpha}$, and the male effect (Ungerfeld and Rubianes, 2002). Intravaginal sponges and CIDR devices are inserted for 12-14 days. The equine chorionic gonadotropin (eCG) is used before, in the moment, or after the sponge or device is removed. Their combination is the most common hormone treatment used to simulate the luteal phase for the synchronization of the estrous (Ataman *et al.*, 2006). In this context, eCG application has been proven to have positive effects on the fertility and productivity of ewes (Ince and Karaca, 2009). The aim of using eCG in progesterone synchronization programs is to increase the ovulation rate and, consequently, to increase the multiple birth rate (Macías *et al.*, 2013). Nevertheless, several factors can modify the reproductive efficiency of ewes, including: breed, seasonality, age, environment, nutrition, diseases, semen quality, reproductive state, hormone treatment, progesterone concentration in plasm, and vaginal microbiota (Mustafa *et al.*, 2007).

Currently, a demand for clean products has arisen and, consequently, the use of chemical and hormone treatments for domestic animals has been reduced or completely avoided (Martin *et al.*, 2004; Eisler *et al.*, 2014). Therefore, researches have focused on developing alternative reproductive methods, which interact both with animal welfare and the environment. Their objective is to improve the reproductive efficiency of ewes (Fierro *et al.*, 2017). Consequently, protocols for the synchronization of the estrous during short periods have been recently established, in order to determine an optimal biological response to a hormone treatment. The aim is to obtain similar results to the conventional treatments regarding the luteal phase simulation, improving pregnancy rates, even during the seasonal anestrous (Ustuner *et al.*, 2007; Özyurtlu *et al.*, 2011; Nasser *et al.*, 2012). Therefore, the objective of this study was to evaluate the effect of the synchronized luteal phase reduction and the application of a low eCG dose on the reproductive efficiency of primiparous and multiparous ewes, during their reproductive season.

MATERIALS AND METHODS

The study was carried out in the sheep unit of the experimental farm of the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico (19° 48' 23" N and 98° 48' 27" W, at 2,241 m.a.s.l). The climate is subhumid warm, with a mean annual precipitation of 632.5 mm and a temperature range of 12 to 18 °C (García, 1988). The animals were handled according to the ethic and biosecurity standards of the Council for International Organizations of Medical Sciences (CIOMS, 1986) and in compliance with the NOM-062-ZOO-1999 Mexican law, regarding the use of animals in experiments (DOF, 2001).

Animals and treatments

Eighty-one Dorset ewes, in their reproductive season, with a 54±4.2 kg average weight, and a body condition score of 3 (scale: 1-5), were used for the experiment. The sheep were previously dewormed with ivermectin; additionally, they were injected with Bayer® ADE vitamin. Additionally, a Sonovet 600 ultrasound scanner (Medison, Inc., Cypress, California, USA) was used to carry out a transrectal ultrasound, in order to determine if

the ewes were pregnant. All the ewes were fed *ad libitum* with oat (*Avena sativa*) hay and 600 g of commercial forage, which included 14% raw protein (RP) and 2.4 Mcal kg^{-1} of metabolizable energy (ME), in compliance with the sheep nutritional requirements (NRC, 2007). They also had *ad libitum* access to water. Based on their reproductive activity, ewes were divided into: primiparous (n=38) and multiparous (n=43). Subsequently, they were randomly further divided into groups for their hormone treatments. Before the sponges were inserted, ewes were pre-synchronized using two doses of prostaglandin $F_{2\alpha}$ (65 mg cloprostenol, Celosil®, Schering-Plough), at 8-day intervals. The first primiparous group (P) was synchronized with cronolone sponges (20 mg, Chronogest®, Intervet) in two periods: 12 days —with (P12+eCG, n=8) and without (P12+0, n=9) the application of 100 UI equine chorionic gonadotropin (eCG; Folligon®, Intervet)— and 6 days —with (P6+eCG, n=9) and without (P6+0, n=12) the application of 100 UI equine chorionic gonadotropin (eCG; Folligon®, Intervet). Both eCG applications were carried out when the sponge was removed. The same 12- and 6-day synchronization program was used for the multiparous ewes (M): M12+0, n=11; M12+eCG, n=12; M6+0, n=11; M6+eCG, n=9.

The detection of the estrous started 24 h after the removal of the sponge, using males with antimating aprons; subsequently, ewes were monitored every 4 h, during 72 h, in order to determine the duration and end of the estrous. Ewes mated at least twice with males of proven fertility, at 12 h intervals. The return of the estrous was detected twice a day (morning and evening), between 15 and 18 days after the mating. The pregnancy was confirmed transrectally 30 days after the mating, using a Sonovet 600 ultrasound scanner and a 7.5 MHz transducer.

Sampling and lab analysis

The 5 mL blood samples were gathered through a puncture in the jugular vein at 08:00 h (from fasting ewes). In order to determine the P_4 concentration in serum, the samples were collected two days before inserting the sponges and, subsequently, every 48 h during the estrous synchronization (12 days). All the samples were centrifuged at 1,500 g at 4 °C for 15 minutes in an IEC Centra 8R (International Equipment Company, USA). The blood serum was separated and stored in polypropylene tubes; subsequently, they were preserved in a freezer at −20 °C, awaiting the hormone analysis. The P_4 analysis was carried out using an enzyme-linked immunosorbent assay (Immunometrics, UK Ltd., 280 Muster Road, London SW6 6BQ). The analytical sensitivity was 0.13 ng mL^{-1}, with an inter- and intra-Assay Coefficients of Variability of 9.59 and 13.7%, respectively.

Statistical Analysis

The experimental design was completely random, with a 2×2×2 factorial arrangement; each ewe was an experimental unit. The main factors were the reproductive physiological state (primiparous and multiparous), the period of protocol of the synchronization of the estrous (6-12 days), and the application of a low eCG dose (0-100 UI). The percentages of the occurrence of the estrous and pregnancy were analyzed using the χ^2 test, through the PROC FREQ. An analysis of variance was used for the start and duration of the estrous, using the PROC GLM and the Tukey's mean comparison test. P_4 concentration

was subjected to an analysis of variance, with repeated measurements throughout time, using the PROC MIXED, which included fixed effects of the treatment and day, as well as their interaction. For this procedure, the covariance structure was modeled using the effect of the sheep inside the group. For this variable, the first-order autoregressive model (AR 1) was used to determine the correlation between sequential measurements within the same animal. The mean values were compared using the least squares method. All the procedures were carried out using the statistical analysis system software suite (SAS, 2009).

RESULTS AND DISCUSSIONS

Occurrence, start, and duration of the estrous

Whether the period of the synchronization of the luteal phase (Table 1) was short or long or regardless of the primiparous (P: 97.9%) or multiparous (M: 95.6%) physical state of the ewe, the estrous was not different ($P>0.05$) between hormone treatments. The response to the start of the estrous was not different ($P>0.05$) between hormone treatments during the synchronization program. However, there were differences caused by the reproductive physiological state of the ewe ($P<0.05$) (Table 1). Differences were found between treatments for the duration of the estrous variable ($P<0.05$) (Table 1). These differences were caused by the effects of the reproductive physiological state (P: 37.2±1.5 h *vs.* M: 41.3±1.4 h) and the duration of the synchronized luteal phase (6 days: 42.2±1.4 h *vs.* 12 days: 36.3±1.5 h of synchronization).

The differences detected on the occurrence of the estrous after a hormone treatment are the consequence of several factors, including breed, season, location, nutrition, weather, and the presence of a male after the removal of the intravaginal sponge (Khalilavi *et al.*, 2016). Nevertheless, the reproductive physiological state of the ewe, the synchronization time, the concentration or dose must be also taken into account before using the different devices or hormone treatments. Other researchers obtained similar results regarding the presence of the estrous, even after the reproductive season. The following synchronization protocols were reported: 6-14 days (Ungerfel and Rubianes, 2002), 6-12 days (Ustuner *et al.*, 2007), and 7 days (Özyurtlu *et al.*, 2011). For their part, Alves *et al.* (2016) reported lower results than those obtained in this experiment: 72-80% estrous responses, using synchronization periods of 6, 9, and 12 days. However, regarding the simulation of the luteal phase, these results only prove that the short period treatment (6 days) with progesterone is as effective as the conventional treatment (12 days). This treatment has an excellent induction to the estrous and, therefore, can be used as an alternative for the synchronization of ewes during their reproductive stage (Özyurtlu *et al.*, 2011).

Several researches report that approximately 90% of the estrous start within a 48-72 interval, after the removal of the sponge or hormone device, combined with low eCG doses (Koyuncu and Altcekic, 2010). Nevertheless, the start of the estrous in this study was caused by the effect of the hormone treatment during the synchronization. In average, the estrous started 35.9±2.3 h for the groups treated for the short period (6 days) and 37.5±1.4 h for the usual period (12 days). However, the estrous occurred earlier than the results reported by Khalilavi *et al.* (2016), who recorded an average of 44.73±4.4 h (6 days) and 45.62±3.76 h (12 days) of synchronization using MAP. The estrous was longer in the

group of multiparous ewes than in the primiparous ewes. There are few comparative studies about the occurrence of the estrous in ewes with different reproductive physiological states. On the one hand, the results of the duration of the estrous obtained with multiparous ewes are higher than the 17.38-27.27 h range reported by Khalilavi *et al.* (2016). On the other hand, the results obtained by the primiparous ewes are similar to the 39-42 h period reported by Alves *et al.* (2016). The duration of the estrous was also influenced by the synchronization period and was longer for the 6 days group than for the 12 days group. These results are similar to those reported by Ustuner *et al.* (2007) and Nasser *et al.* (2012).

Hormone profile of progesterone (P_4)

P_4 concentrations in plasm were different ($P<0.05$) during the synchronized luteal phase and after the estrous. The results obtained in this experiment reflected a higher concentration in primiparous ewes (1.8 ± 0.1 ng mL^{-1}) than in multiparous ewes (0.9 ± 0.1 ng mL^{-1}), showing a quick increase in the P_4 concentrations, 2 days after the removal of the sponge of the new estrous cycle (Figure 1). Bartlewski *et al.* (1999) established that P_4 concentrations higher than 1 ng mL^{-1} are the result of a functional corpus luteum at the moment that the sponge is inserted. Additionally, the insertion of the sponge soaked with progesterone does not affect the P_4 production by the corpus luteum. Only when the sponge is inserted at the beginning of the luteal phase, the production and its average life could be affected, because the corpus luteum does not secrete enough P_4 during this stage for a normal average life. However, it makes it susceptible to an $PGF_{2\alpha}$αearly secretion injury (Ainsworth, 1985). Nevertheless, the primiparous ewes' group had a higher P_4 concentration in plasm. These results match the findings of Husein and Kridli (2002), Husein and Haddad (2006), and Khalilavi *et al.* (2016), who reported that the P_4 levels increased 2 days after the insertion of the sponge and diminished when the sponge was removed, driving the estrous behavior. However, the P_4 concentrations in the primiparous ewes again had a sudden increase during the next estrous cycle (Figure 1). These results

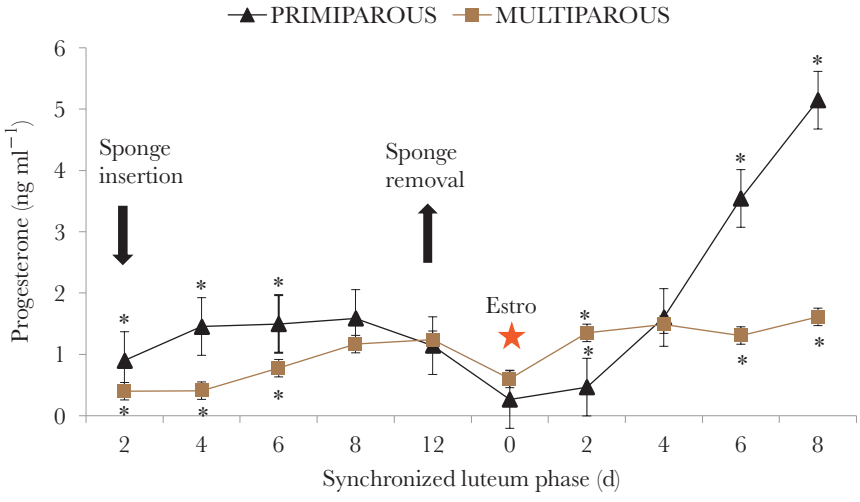

Figure 1. Concentration of progesterone in plasm (mean ± standard error) during synchronized luteum phase in primiparous and multiparous ewes. * Indicates statistical difference ($P<0.05$) between experimental groups.

suggest that these ewes can continue to develop bigger corpus luteum or that their metabolism is not capable of diminishing its concentration in blood plasm, unlike the multiparous ewes.

Pregnancy and prolificity rates

Neither the synchronization period and the eCG application, nor the reproductive physiological state of the ewe, resulted in differences ($P>0.05$) in the pregnancy and prolificity rates. The pregnancy rate of the ewes at 30 days after the mating was 95.3% and 97.3% for the multiparous and the primiparous groups, respectively. Likewise, the multiparous group recorded a 1.26 index, while the primiparous group had a 1.29 index (Table 1).

The pregnancy percentages obtained in this study are similar to those reported by Mustafa *et al.* (2007), who recorded a 91.6% pregnancy among ewes. However, our results are higher than those obtained by Ali (2007), who reported 91.6% pregnancies, using 40 mg of FGA for 8 days and 500 UI of eCG for two periods (48 h before and at the moment of the removal of the sponge). Ali concluded that the use of eCG improves the ovulation rates, but not the pregnancy percentage.

In this regard, Alves *et al.* (2016) reported 45.5, 36.4, and 20% pregnancy rates for 6-, 9-, and 12-days synchronization protocols, respectively. These synchronizations were carried out with ewes inseminated by laparoscopy. In contrast, Özyurtlu *et al.* (2011) reported 58.3-66.7% pregnancy rates during an anestrous period, applying 500 UI of eCG to ewes with 7-14 days synchronization. Likewise, Khalilavi *et al.* (2016) reported 66.6-80% pregnancy rates, as a result of the application of 300 UI of eCG to ewes with 6-12 days synchronization. In both studies, the ewes were directly mated with studs of proven fertility. There was no difference in the prolificity index between treatments. Nevertheless, our results are similar to those reported by Mustafa *et al.* (2007), who used 500 UI of eCG and obtained 1.18 and 1.11 prolificity with hormone treatments of 12 and 14 synchronization days, respectively. The use of eCG in the estrous synchronization protocols has an important effect on the ovulation rate of the ewe and, therefore, in its prolificity. According to Koyuncu and Ozis (2010), the application of 500 UI of eCG should take place 24 h before or at the moment of the removal of the sponge. Additionally, the subcutaneous application of eCG has better results for multiple lambings and fecundity than the intramuscular application of eCG. In this research, the application of 100 UI of eCG sought to support the follicular growth, in order to induce the estrous, mainly in the primiparous group. Quintero *et al.* (2011) obtained similar results in hair sheep: 1.8, 1.9, and 2.2 prolificity, with the application of 100, 200, and 400 UI of eCG, respectively. Likewise, Macias *et al.* (2013) pointed out that low doses of eCG (140-280 UI) can induce the estrous in hair sheep, even under heat stress. They obtained 1.8 (140 UI) and 2.2 (280 UI) prolificity rates.

Overall, the pregnancy percentage and the prolificity index obtained in this study are acceptable, regarding the hormone treatments used. These results suggest a development of the corpus luteum and an increase of the secreted progesterone, which was required to provide the endometrium with the appropriate conditions for the implantation of the embryo and the preservation of the pregnancy.

Table 1. Reproductive response variables in primiparous (P) and multiparous (M) ewes during short (6 d) and long (12 d) periods of synchronization with low dose of equine chorionic gonadotropin (eCG).

Reproductive Variables	Multiparous				Primiparous			
	M12+eCG (n=12)	**M12+0 (n=11)**	**M6+eCG (n=9)**	**M6+0 (n=11)**	**P12+eCG (n=8)**	**P12+0 (n=9)**	**P6+eCG (n=9)**	**P6+0 (n=12)**
Estrus response (%)	100 (12/12)	100 (11/11)	100 (9/9)	100 (11/11)	100 (8/8)	100 (9/9)	100 (9/9)	91.6 (11/12)
Estrus onset (h) [†1]	33±4.5[a]	35.2±3.2[a]	30.6±4.6[a]	37.4±2.4[a]	40.5±1.8[b]	41.3±2.4[b]	37.3±1.5[a]	38.3±1.4[a]
Estrus duration (h)	34±1.4[b]	40.3±1.4[ab]	48±1.5[a]	42±1.4[ab]	34±1.5[b]	36±1.5[ab]	40±1.5[ab]	38±1.4[ab]
Gestation (%)[2]	91.6 (11/12)	100 (11/11)	100 (9/9)	90.9 (10/11)	100 (8/8)	100 (9/9)	100 (9/9)	91.6 (11/12)
Prolificity index	1.27±0.1	1.27±0.1	1.30±0.1	1.20±0.1	1.25±0.1	1.33±0.1	1.44±0.1	1.18±0.1

1 Time after sponge removal.
2 Based on P_4 profiles in serum and ultrasound on day 30.
3 Number of lambs born per ewe.
[a, b] Values with different letters in columns are different ($P<0.05$).
[†] Means±standard error.

CONCLUSIONS

The combination of intravaginal cronolone sponges with 100 UI of eCG in short (6 days) and long (12 days) synchronization periods favors the occurrence of the estrous in primiparous and multiparous ewes, during the mating season. However, the variations of the start and duration of the estrous are caused by the reproductive physiological state of the ewe (primiparous and multiparous) and by the duration of the synchronized luteal phase (6 and 12 days). Consequently, these results must be taken into account when artificial insemination takes place in a fixed time. Finally, the P_4 concentrations in serum were higher in primiparous ewes, although this result did not determine a pregnancy and prolificity increase in the treated ewes.

ACKNOWLEDGEMENTS

The authors would like to thank the Consejo Nacional de Ciencia y Tecnología (Conacyt) for its support to the Programa de Estancias Posdoctorales por México and its commitment to scientific and technological research for the benefit of society.

REFERENCES

Ainsworth, L. (1985). Effects of norgestomet-implants and fluorgestone acetate-impregnated sponges on oestrus cycle length and luteal function of ewes. *Anim. Reprod. Sci*. 9: 63-73. doi.org/10.1016/0378-4320(85)90042-9.

Ali, A. (2007). Effect of time of eCG administration of follicular response and reproductive performance of FGA – treated Ossimi ewes. *Small Rum. Res*. 72: 33-37. doi.org/10.1016/j.smallrumres.2006.07.017.

Alves, T.T., Ferreira, F.J., Goncalves, S.J., Rezende, C.L., Moura, F.D., & Zandonadi, B.F. (2016). Efficiency of different hormonal treatments for estrus synchronization in tropical Santa Inês sheep. *Trop. Anim. Heal. Prod*. 48: 545 – 551. doi.org/10.1007/s11250-015-0989-y.

Bartlewski, P.M., Beard, A.P., & Rawling, C.N. (1999). An ultrasonographic study of luteal function in breeds of sheep with different ovulation rates. *Theriogenology*. 52: 115-130. doi.org/10.1016/S0093-691X(99)00114-4.

CIOMS (Council for international Organizations of Medical Sciences). (1986). "International Guiding Principles for Biomedical Research Involving Animals". CIOMS, Geneva, Switzerland.

DOF (Diario Oficial de la Federación). (2001). "Norma Oficial Mexicana NOM-062-ZOO-1999: Especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio". México, D.F.

Eisler, M.C., Lee, M.R.F., Tarlton, J.F., Martin, G.B., Beddington J., Dungait, J.A.J., Greathead, H., Liu, J., Mathew, S., Miller H., Misselbrook, T., Murray, P., Vinod, V.K., Van Saun, R., & Winter M. (2014). Steps to sustainable livestock. *Nature* (London). 507: 32 – 34. doi.org/10.1038/507032a.

Fierro, S., Viñoles, C., & Olivera, M.J. (2017). Long term prostaglandin based – protocols improve the reproductive performance after timed artificial insemination in sheep. *Therio. Inter. Jour. Anim. Reprod*. 90: 109 – 113. doi.org/10.1016/j.theriogenology.2016.11.031.

García, E. (1988). Distribución de los grupos climáticos de Köppen en México. Modificaciones al sistema de clasificación climática de Köppen (Para adaptarlo a las condiciones de la República Mexicana). Primera parte. Instituto de Geografía. Universidad Nacional Autónoma de México. 4 edición. México D.F. 217 pp.

Husein, M.Q., & Haddad, S.G. (2006). A new approach to enhance reproductive performance in sheep using royal jelly in comparison with equine chorionic gonadotropin. *Anim. Reprod*. Sci. 93: 24 – 33. doi.org/10.1016/j.anireprosci.2005.06.012.

Husein, M.Q., & Kridli, R.T. (2002). Reproductive response of Awassi ewes treated with either naturally occurring progesterone or synthetic progestagen. *J. Anim. Sci. 15*(9): 1257 – 1262. doi.org/10.5713/ajas.2002.1257.

Ince, D., & Karaca, O. (2009). Effects of oestrus synchronization and various doses of PMSG administrations in Chios x Kivircik (F1) sheep on reproductive performances. *J. Anim. Vet. Adv*. 8: 1948 – 1952.

Khalilavi, F., Mamouei, M., Tabatabaei, S., Chaji, M., 2016. Effect of different progesterone protocol and low doses of equine chorionic gonadotropin (eCG) on oestrus synchronization in Arabian ewes. Iran. *Jour. App. Anim. Sci. 6*(4), 855-861.

Koyuncu, M., & Alticekic, S. (2010). Effects of progesterone and PMSG on estrous synchronization and fertility in Kivircik ewes during natural breading season. Asian-Aust. *J. Anim.* 23: 308 – 311. doi.org/10.5713/ajas.2010.90393.

Macías, C.U., Ponce, C.J.L., Álvarez, V.F.D., Correa, C.A., Meza, H.C.A., & Avendaño, R.L. (2013). Reproductive efficiency of pelibuey and Romanov × pelibuey ewes synchronized with synthetic progesterone and low doses of pmsg under a hot environment. *Czech. J. Anim. Sci*. 12: 546-553.

Martin, G.B., Milton, J.T.B., Davidson, R.H., Banchero-Hunzicker, G.E., Lindsay, D.R., & Blache, D. (2004). Natural methods for increasing reproductive efficiency in small ruminants. *Anim. Reprod. Sci*. 82: 231 – 245. doi.org/10.1016/j.anireprosci.2004.05.014.

Mustafa, Q.H., Ababneh, M.M., & Abu-ruman, D.S. (2007).The effects of short or long term FGA treatment with or without eCG on reproductive performance of ewes bred out-of-season. *Am. J. Anim. Vet. Sci. 2*(1): 23-28.

Nasser, S.O., Wahid, H., Aziz, A.S., Zuki, A.B., Azam, M.K., Jabbar, A.G., & Mahfoz, M.A. (2012). Effect of different oestrus synchronization protocols on the reproductive efficiency of dammar ewes in yemen during winter. *Afri. J. Biotec. 11*(37): 9156-9162. 10.5897/AJB12.265.

National Research Council (NRC). (2007). Nutrient requirements of small ruminants. Sheep, goats, cervids and new world camelids. National Academy Press, Washington D.C.

Özyurtlu, N., Ay, S.S., Kücükaslan, I., Güngör, Ö., & Aslan, S. (2011). Effect of subsequent two short – term, short – term, and long – term progestogen treatments on fertility of awassi ewes out of the reproductive season. Ankara Üniv. *Vet. Fak. Derg*. 58: 105-109.

Quintero, E.J., Macías, C.U., Alvarez, V.D., Correa, C.A., González, R.A., Lucero, M.F., Soto, N.S., & Avendaño, R.L. (2011). The effects of time and dose of pregnant mare serum gonadotropin (PMSG) on reproductive efficiency in hair sheep ewes. *Trop. Anim. Health. Prod*. 43: 1567 – 1573. doi.org/10.1007/s11250-011-9843-z.

Statistical Analysis System Institute (SAS). (2009). SAS User's Guide: Statistics (Version 5). Cary, N.C. U.S.A. Inst. Inc. 584 pp.

Ungerfeld, R., & Rubianes, E. (2002). Short-term priming with different progestogen intravaginal devices (MAP, FGA, and CIDR) for eCG-estrous induction in anestrus ewes. *Small Rumin. Res*. 46: 63 – 66. doi.org/10.1016/S0921-4488(02)00105-0.

Ustuner, B., Gunay, U., Nur, Z., & Ustuner, H. (2007). Effects of long and short-term progestogen treatments combined with PMSG on oestrus synchronization and fertility in awwasi ewes during the breading season. *Act. Vet. Brno*. 76: 391-397. doi:10.2754/avb200776030391.